

are readily assigned to the  $\gamma$ -methylene and methyl carbons by comparison with the liquid-phase spectrum. These resonances are only slightly shifted upon adsorption. The remaining four resonances arise from the two  $\alpha$ - and  $\beta$ -methylene carbons, from which we conclude that a least two types of chemically different butylamine species are present on the surface.

The existence of well-resolved resonances demonstrates that if surface diffusion is occurring, then the rate is not comparable to the spinning rate ( $\sim 3.2$  kHz).<sup>13</sup> Further, if site exchange is occurring, then the rate must be slow compared to the chemical-shift difference, between the two species on the surface. Therefore, this spectrum is consistent with a picture whereby the nitrogen of the *n*-butylamine is firmly anchored to the surface. In all probability the system is executing rapid albeit limited angular diffusion about the bond axis connecting the surface to the nitrogen, with the motion of the alkyl chains increasing as one moves away from the surface.

The appearance of four resonances in the region expected for the two  $\alpha$  and  $\beta$  carbons of the alkyl group is consistent with there being two quite different sites available to the amine. Two candidates are the classic Lewis and Brønsted sites. In order to check this possibility we obtained the CP-MAS <sup>13</sup>C spectra of two solid adducts of *n*-butylamine (Figure 1b,c). Those resonances for the solid HCl adduct match closely to four of the resonances for the surface adsorbed species. The resonances corresponding to the  $\alpha$  and  $\beta$  carbons of the solid BCl<sub>3</sub> adduct are deshielded relative to the corresponding carbons within the HCl adduct but shielded relative to the analogous carbons of the adsorbed amine. Hence, we conclude that the most deshielded set of resonances correspond to the *n*-butylamine which is attached to the surface via Lewis bonds to an aluminum atom. That the chemical shifts of this species are deshielded relative to those of the BCl<sub>3</sub> adduct we attribute to the fact that the acid site in this surface is a stronger Lewis acid than BCl<sub>3</sub>.

The breadth of the resonances for the  $\alpha$  and  $\beta$  carbons may arise from several factors. An important consideration for the  $\alpha$  carbon is the presence of nitrogen-14 dipolar coupling. The fact that we do not observe "well-resolved" doublets<sup>14</sup> for this carbon may be due in part to the angular diffusion of the molecule and/or the strength of the applied magnetic field relative to the nitrogen-14 quadrupolar coupling constant. Perhaps of equal importance to the line width is a heterogeneity of the acid sites on the surface of the alumina. The presence of a distribution of site acidities would lead to a corresponding distribution of chemical shifts and hence appear as a line-broadening mechanism.

In summary, it is clearly evident that <sup>13</sup>C NMR spectroscopy in conjunction with CP-MAS methods can be used to probe the interactions between small molecules and surfaces even when the surface is of moderately low specific area. Compared to other spectroscopies carbon-13 CP-MAS NMR spectroscopy appears to provide a facile method to distinguish between different types of surface bonding sites and their relative acidity. Within the limitations of cross-polarization dynamics the relative populations of these sites can be quantified.

(10) The  $\gamma$ -alumina (SA = 220 m<sup>2</sup>/g) employed in this study was pretreated at 350 °C in vacuum. The *n*-butylamine was laid down on the surface by using entrainment in a helium flow. The basis of a weight increase of approximately 0.5%, the surface covered by the butylamine is estimated to be 10 m<sup>2</sup>/g or roughly one-twentieth of a monolayer. Operating under a dry nitrogen atmosphere the granular alumina sample was packed into an aluminum oxide NMR rotor.

(11) An important experimental difficulty that had to be overcome with these relatively dilute samples (typically 40 mM in amine based on the total volume of the NMR rotor) was the carbon background signal from the NMR probe. We accomplished this using sample machined from aluminum oxide and a stator from Macor. The Delrin end caps of the rotor leave a small signal at 90 ppm which we use for reference.

(12) (a) Brown, T. D. Ph.D. Thesis, University of Utah, 1965. (b) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 152.

(13) Maricq, M. M.; Waugh, J. S. *J. Chem. Phys.* 1979, 70, 3300.

(14) (a) Hexem, J. G.; Frey, M. H.; Opella, S. J. *J. Am. Chem. Soc.* 1981, 103, 224. (b) Frey, M. H.; Opella, S. J. *J. Chem. Soc., Chem. Commun.* 1980, 474. (c) Groombridge, C. J.; Harris, R. K.; Packer, K. J.; Say, B. J.; Tanner, S. F. *J. Chem. Soc., Chem. Commun.* 1980, 174.

**Acknowledgment.** We thank Z. Szafran for his assistance in preparing the amine and F. D. Doty for the MAS probe design used for this work. We are indebted to Dr. R. C. Schoening for his useful advice. The encouragement and support of the management of Union Carbide Corporation is gratefully acknowledged. The use of the facilities at the University of South Carolina Regional NMR Center, funded by the National Science Foundation Grant CHE78-18723, is acknowledged.

## Laser Photochemical Production of Vitamin D<sup>†</sup>

V. Malatesta,\* C. Willis, and P. A. Hackett

Laser Chemistry Group, Division of Chemistry  
National Research Council of Canada  
Ottawa, Ontario, Canada K1A 0R6

Received October 27, 1980

The complex photochemistry of 7-dehydrocholesterol (7-DHC) and ergosterol (E) is now moderately well understood due, in large part, to the elegant studies of Havinga and co-workers.<sup>1-9</sup> Table I shows how the distribution of products depends on photolyzing wavelength.<sup>10</sup> Photolysis in the range 270–310 nm gives relatively high yields of previtamin D (P); shorter wavelengths lead to a predominance of tachysterol (T) and longer wavelengths to lumisterol (L).<sup>11</sup> The interrelations between the various products are shown in Figure 1.

Using laser photolysis, we have confirmed the main aspects of the dependence of the product array on wavelength and have gone further by demonstrating that by a two-stage photolysis we can "engineer" a desirable product distribution with improved yields. This approach may offer an attractive alternative to the commercial method of vitamin D production, which requires a number of fractional crystallizations.<sup>15</sup>

Photolysis at KrF (248 nm) and XeCl (308 nm) wavelengths was carried out with an excimer laser (Lumonics Research Limited, Model 861), which gives average powers in the 1–10-W range. Photolysis at 337 nm used a nitrogen laser (Molelectron, Model UV 1000) with an average power of 0.2 W while at 353 nm a YAG laser (Molelectron, Model MY 34) with an average power of 0.4 W was used. The reaction mixtures were analyzed by high-performance LC<sup>20</sup> (Varian 8500) on a silica Si-5 column (5

<sup>†</sup> Issued as NRCC 18928.

(1) Koevoet, A. L.; Verloop, A.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1955, 74, 788.

(2) Verloop, A.; Koevoet, A. L.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1955, 74, 1125.

(3) Havinga, E.; de Kock, R. J.; Rappoldt, M. P. *Tetrahedron* 1960, 11, 276.

(4) Rappoldt, M. P.; Keverling Buisman, J. A.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1958, 77, 321.

(5) Rappoldt, M. P. *Recl. Trav. Chim. Pays-Bas* 1960, 79, 392.

(6) Rappoldt, M. P.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1960, 79, 369.

(7) Havinga, E.; Koevoet, A. L.; Verloop, A. *Recl. Trav. Chim. Pays-Bas* 1955, 74, 1230.

(8) Havinga, E. *Chimie* 1976, 30, 27.

(9) Bousma, F.; Jacobs, H. J. C.; Havinga, E.; Van der Gen, A. *Tetrahedron Lett.* 1975, 7, 427.

(10) (a) Although the composition of the photostationary state reported in Table I refers to the photolysis of ergosterol, we believe that it is fair to compare with the photolysis of 7-DHC because of the chromophoric similarity. (b) Kobayashi, T.; Yasumura, M. T. *Vitaminology* 1972, 18, 78. *J. Nutr. Sci. Vitaminol.* 1973, 19, 123.

(11) Havinga, E.; Bots, P. *Recl. Trav. Chim. Pays-Bas* 1954, 73, 393.

(12) Pfoertner, K.; Weber, J. F. *Helv. Chim. Acta* 1972, 55, 921.

(13) Pfoertner, K. *Helv. Chim. Acta* 1972, 55, 937.

(14) Yakimovich, R. E.; Vendt, V. P.; Boguslavskii, V. A.; Prikl. Biokhim. *Mikrobiol.* 1975, 11, 254.

(15) Bharucha, K. R.; Martin, F. M. French Patent 1 378 122, 1964.

(16) Havinga, E. *Experientia* 1973, 29, 1181.

(17) The cis-trans isomerization of the C<sub>67</sub> double bond can be brought about by triplet sensitization<sup>18,19</sup> to give a photostationary state (80% P<sub>2</sub>; 20% T<sub>3</sub>).

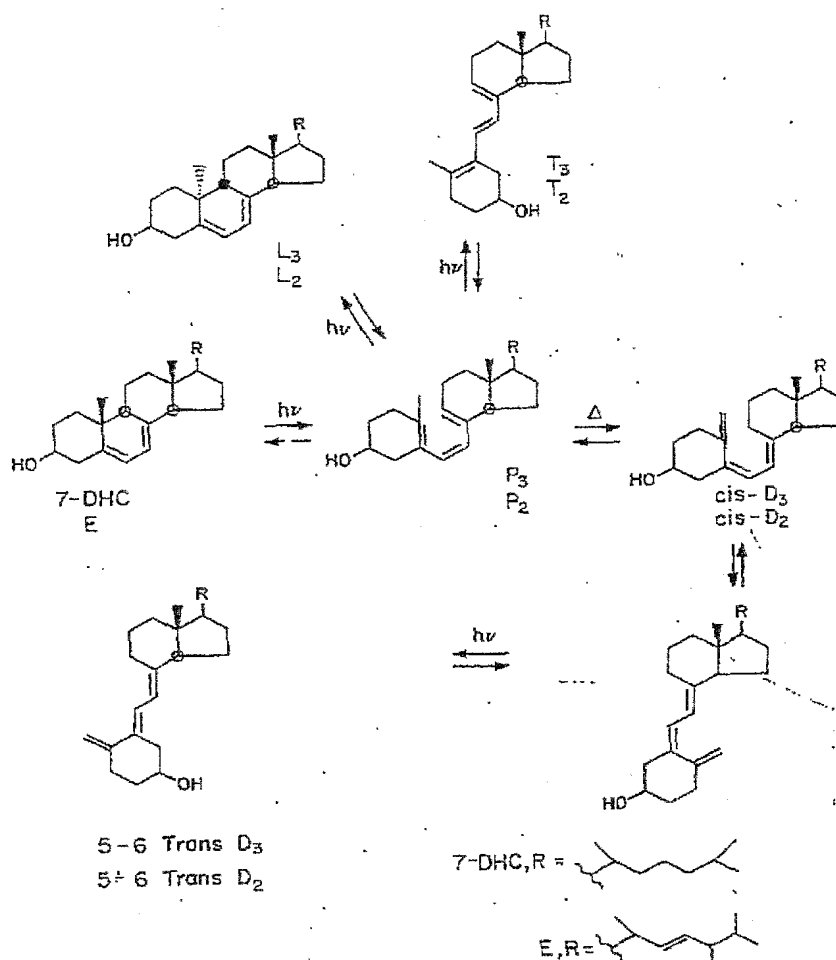


Figure 1.

Table I. Composition of the Photostationary State at Various Wavelengths

wavelength/ nm	% E <sup>a</sup>	% P <sub>3</sub> <sup>b</sup>	% T <sub>2</sub> <sup>c</sup>	% L <sub>2</sub> <sup>d</sup>	% D <sub>2</sub> <sup>e</sup>	ref <sup>f</sup>
248	2.9 <sup>a</sup>	25.8 <sup>b</sup>	71.3 <sup>c</sup>	nd		this work
254	1.5	20	75	2.5		16
302	3.4	53	26	17		14
308	13.3 <sup>a</sup>	35.5 <sup>b</sup>	3.41 <sup>c</sup>	42.3 <sup>d</sup>	4.5 <sup>e</sup>	this work
(248 + 337) <sup>f</sup>	8.8 <sup>a</sup>	79.8 <sup>b</sup>	1.5 <sup>c</sup>	9.8 <sup>d</sup>		this work <sup>g</sup>
(248 + 353) <sup>f</sup>	0.1 <sup>a</sup>	80.1 <sup>b</sup>	11.0 <sup>c</sup>	8.7 <sup>d</sup>		this work

<sup>a</sup> 7-DHC. <sup>b</sup> P<sub>3</sub>. <sup>c</sup> T<sub>2</sub>. <sup>d</sup> L<sub>2</sub>. <sup>e</sup> cis-D<sub>2</sub>. <sup>f</sup> Photolyzed to a photostationary state at 248 nm and then photolyzed at the second wavelength. Because of the long photolysis times (95 min at 337 nm and 180 min at 353 nm), complete photoequilibrium might not have been attained. <sup>g</sup> A better separation between P<sub>3</sub> and L<sub>2</sub> is obtained when a mixture of n-hexane and n-amyl alcohol (0.3%) is used as eluent. (M. P. Rappoldt, personal communication).

μm) at a flow rate of 0.5 cm<sup>3</sup> min<sup>-1</sup> under an operating pressure of 90 atm. The eluent used was 70:30:2 chloroform-hexane-ethyl acetate and the compounds were identified by UV monitoring at 282 nm. The relative concentration was calculated from relative area; after allowance was made for extinction coefficient at the monitoring wavelength.

(18) Eyley, S. C.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* 1975, 858.

(19) Snoeren, A. E. C.; Dahan, M. R.; Lugtenburg, J.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1970, 89, 261.

(20) The same product sequence was obtained on a silica gel TLC plate eluted with the LC eluent (P<sub>3</sub>, R<sub>f</sub> 0.36; T<sub>2</sub>, R<sub>f</sub> 0.24; 7-DHC, R<sub>f</sub> 0.15; cis-D<sub>2</sub>, R<sub>f</sub> 0.17).

The photoproduct yields we observe in single-wavelength photolysis are included in Table I and agree with the general features previously established. Irradiation at 248 nm of a  $6.4 \times 10^{-5}$  M solution of 7-DHC in O<sub>2</sub>-free diethyl ether (0 °C) distilled from benzophenone ketyl gives a conversion of about 96% (15 W-min incident) with L<sub>2</sub> below our limits of detection. This agrees with the results of Pfoertner,<sup>12,13</sup> who found that a solution of ergosterol irradiated at 254 nm gave only T<sub>2</sub>. Similarly, the observations at 308 nm agree with those of Yakhimovich et al.,<sup>14</sup> who, using a lamp with maximum output at 302–305 nm, showed it was possible to minimize the amount of T<sub>3</sub> when the conversion of 7-DHC is kept to 40–45%. The yields of T<sub>3</sub> and L<sub>2</sub> after 248-nm photolysis support the scheme proposed by Havinga,<sup>16</sup> where, of the three possible photoproducts from previtamin D<sub>2</sub> (T<sub>2</sub>, E, and L<sub>2</sub>), only T<sub>2</sub> has a significant forward quantum yield whereas the other two have high reverse quantum yields.

Significant improvement in overall yield and decrease in side-product contamination is possible by two-stage laser photolysis. Taking the mixture produced by photolysis with the KrF laser at 248 nm (25.8% P<sub>3</sub>, 71.3% T<sub>2</sub>, 2.9% 7-DHC), photolysis with an N<sub>2</sub> laser (14 W-min, 337 nm, at room temperature) isomerizes T<sub>2</sub> to previtamin D<sub>2</sub><sup>17</sup> (79.8% P<sub>3</sub>, 1.5% T<sub>2</sub>, 8.8% 7-DHC, 9.8% L<sub>2</sub>). At this wavelength a small amount of P<sub>3</sub> is photolyzed back to 7-DHC and L<sub>2</sub>. A series of weak absorption bands of the cZc triene (P<sub>3</sub>) which occur around 337 nm ( $\epsilon$  60) may account for the cycloaddition reactions P<sub>3</sub> → 7-DHC and P<sub>3</sub> → L<sub>2</sub>.

At longer wavelength T<sub>3</sub> is the only compound having appreciable absorption ( $\epsilon$  175 at 350 nm) and the E → Z isomerization of the C<sub>6,7</sub> double bond of the cEc triene (T<sub>3</sub>) should be the only photoreaction taking place. Indeed room-temperature photolysis with the third harmonic of a YAG laser smoothly converts T<sub>3</sub> to P<sub>3</sub> with minimization of the ring closure to 7-DHC and L<sub>2</sub> (P<sub>3</sub>

80.1%,  $L_3$  11.0%, 7-DHC 0.1%,  $L_3$  8.7%).

Thus by two-stage photolysis using narrow spectral width laser sources it is possible to greatly reduce or eliminate competing photoreactions leading to photoequilibria of complex composition and to have high conversion of 7-DHC (>90%) to  $F_3$  with very low final yields of  $T_3$  and  $L_3$ .

**Acknowledgment.** We wish to express our thanks to Drs. N. Thompson and K. Pfoertner for their helpful suggestions. Samples of tachysterol<sub>3</sub>, 4-methyl-3,5-dinitrobenzoate, lumisterol<sub>3</sub>, and lumisterol<sub>2</sub> were generous gifts of Dr. K. Pfoertner (Hoffmann-La Roche & Co. Ltd., Basel, C.H.) and Dr. M. P. Rappoldt (Duphar Co., Weesp, N.L.).

### Spinning Near the Magic Angle: A Means of Obtaining First-Order Dipolar NMR Spectra of Molecules Dissolved in Nematic Liquid Crystals

Jacques Courtieu,<sup>†</sup> D. W. Alderman, and David M. Grant\*

Department of Chemistry, University of Utah  
Salt Lake City, Utah 84112

Received August 6, 1981

Chemical applications of the dipolar NMR spectra obtained from molecules dissolved in nematic crystals are limited by the difficulty of solving the complicated multispin second-order patterns which arise from the large values of the partially averaged dipolar interactions. As a consequence the only spectra which have been interpreted have been those from molecules either with a small number of resonating nuclei or with high symmetry. Large or nonsymmetrical molecules have not been successfully studied with this otherwise powerful method of determining the molecular geometry.

This communication reports a technique which obtains first-order spectra from such systems by reducing the averaged dipole coupling by any factor up to 100. Such reduction is accomplished by making the angle  $\alpha$  between the nematic director and the magnetic field any value between 0° and the magic angle, 54.73°, at which point the averaged dipole interaction is null. This is accomplished by spinning the sample at a moderate speed (ca. 50 Hz) about an axis which makes an angle less than the magic angle with the magnetic field. The director aligns along the spinning axis instead of along the magnetic field if the rate of spinning exceeds the rate of director reorientation. Large reductions in the dipole interaction are obtained as  $\alpha$  approaches the magic angle. When the director of the liquid crystal is aligned at an angle  $\alpha$  with the magnetic field all the tensor contributions (e.g., the dipole-dipole interaction, the chemical shift anisotropy, the anisotropy of the scalar spin-spin coupling, and the quadrupolar coupling for nuclei with spins greater than  $1/2$ ) in the Hamiltonian are reduced<sup>1,2</sup> by a factor  $R = (1/2)(3 \cos^2 \alpha - 1)$ .

Previous attempts to orient the director of a nematic liquid crystal at a variable angle from the magnetic field have not successfully approached the magic angle. The use of electric fields<sup>3,4</sup> apparently presents serious technical problems which have limited their utility to values of  $\alpha$  near 0 and 90°. Spinning the sample below a critical rotation rate around an axis perpendicular to the magnetic field has also been used to change the director

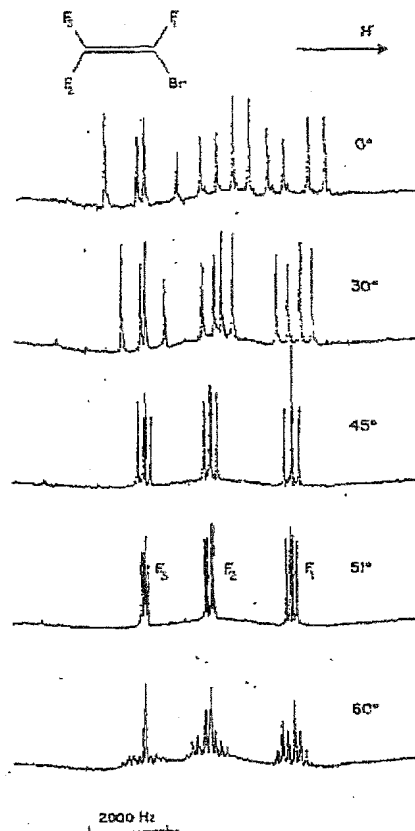


Figure 1.  $^{19}\text{F}$  spectra of  $\text{CF}_2=\text{CFBr}$  in liquid crystal as a function of the angle between the axis of rotation and the magnetic field. Spinning rate 70 rps.

orientation<sup>5,6</sup> from 0 to 45° from the magnetic field,<sup>2</sup> thus missing the magic angle by almost 10°. Furthermore, at rotation speeds which should produce angles larger than 30°, the director is distributed in such a way that the line widths become large. All previous work on rotating liquid crystals has used two particular orientations of the spinning axis relative to the field, viz, 90° in classical electromagnets and 0° in superconducting magnets. Thus, the very interesting region about the magic angle and between these two limiting cases has been neglected.

For this work a spinner has been constructed for 10-mm NMR tubes with a length of 23 mm. The sample spins at frequencies between 20 and 100 Hz around an axis which is horizontal. The spinning axis may be rotated around the vertical so as to make any angle with the horizontal magnetic field of our wide-gap electromagnet.

Fluorine-19 spectra obtained at 75.25 MHz on trifluorobromoethylene dissolved in the liquid crystal *p*-pentyphenyl 2-chloro-4-(*p*-pentybenzoyloxy)benzoate are shown in Figure 1. It is quite apparent that a second-order spectrum of the ABC type is obtained when spinning the sample parallel to the field, spinning about an axis which is 3° less than the magic angle spectrum is clearly of the AMX type. The couplings are dominated by the large dipolar terms, but they are now with the scalar interactions. Note that resolution in the sample is oriented closer to the magic angle. V than the magic angle, a new pattern is observed. the director of the liquid crystal no longer reaches steady state but instead distributes in the plane the spinning axis. The result is a frequency mod-

<sup>†</sup> Permanent address: Laboratoire de Chimie Organique Structurale, Université Paris Sud, 91405 Orsay, France.

(1) Snyder, L. C. *J. Chem. Phys.* 1965, 43, 4041.

(2) Emsley, J. W.; Lyndon, J. C. In "NMR Using Liquid Crystal Solvents"; Pergamon Press: Oxford, 1975; Chapter 9.

(3) deGennes, P. G. "The Physics of Liquid Crystals"; Pergamon Press: Oxford, 1974.

(4) Diehl, P.; Khetrapal, C. L.; Kellerhals, H. P.; Lienhard, U.; Niederberger, W. *J. Magn. Reson.* 1969, 1, 527.

(5) Carr, S. G.; Luckhurst, G. R.; Poupo, R. and Sn *Phys.* 1975, 7, 278.

(6) Emsley, J. W.; Lyndon, J. C.; Luckhurst, G. R.; *J. Lett.* 1973, 19, 345.

respectively) had to be reinjected.

(23R,24R)-4 $\alpha$ ,23,24-Trimethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol (1a): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 430.4179 (C<sub>30</sub>H<sub>54</sub>O, M<sup>+</sup>, 22), 415.3941 (C<sub>29</sub>H<sub>51</sub>O, 16), 412.4089 (C<sub>30</sub>H<sub>52</sub>, 6), 397.3844 (C<sub>29</sub>H<sub>49</sub>, 15), 341.3194 (C<sub>25</sub>H<sub>41</sub>, 3), 303.3067 (C<sub>22</sub>H<sub>39</sub>, 2), 299.2745 (C<sub>22</sub>H<sub>35</sub>, 2), 290.2988 (C<sub>21</sub>H<sub>38</sub>, 7), 271.2433 (C<sub>20</sub>H<sub>31</sub>, 8), 262.2300 (C<sub>18</sub>H<sub>30</sub>, 8), 247.2056 (C<sub>17</sub>H<sub>27</sub>O, 38), 229.1957 (C<sub>17</sub>H<sub>25</sub>, 57), 179.1432 (C<sub>12</sub>H<sub>19</sub>O, 38), 98.1094 (C<sub>7</sub>H<sub>14</sub>, 100).

(23S,24R)-23,24-Dimethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol (2b): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 416.4019 (C<sub>29</sub>H<sub>52</sub>O, M<sup>+</sup>, 33), 401.3761 (C<sub>28</sub>H<sub>49</sub>O, 17), 398.3945 (C<sub>29</sub>H<sub>50</sub>, 9), 383.3683 (C<sub>28</sub>H<sub>47</sub>, 13), 359.3289 (C<sub>25</sub>H<sub>43</sub>O, 4), 344.3456 (C<sub>25</sub>H<sub>44</sub>, 4), 327.3048 (C<sub>24</sub>H<sub>39</sub>, 4), 317.2857 (C<sub>22</sub>H<sub>37</sub>O, 3), 290.2978 (C<sub>21</sub>H<sub>38</sub>, 6), 285.2584 (C<sub>21</sub>H<sub>35</sub>, 5), 257.2268 (C<sub>19</sub>H<sub>29</sub>, 14), 248.2140 (C<sub>17</sub>H<sub>25</sub>O, 11), 233.1904 (C<sub>16</sub>H<sub>25</sub>O, 64), 215.1802 (C<sub>16</sub>H<sub>23</sub>, 74), 165.1275 (C<sub>11</sub>H<sub>17</sub>O, 42), 98.1091 (C<sub>7</sub>H<sub>14</sub>, 100).

(23R,24R)-4 $\alpha$ ,23,24-Trimethyl-5 $\alpha$ -cholestan-3-one (6a): high-resolution MS (70 eV, probe), *m/z* (assignment, relative intensity) 428.4029 (C<sub>30</sub>H<sub>52</sub>O, M<sup>+</sup>, 25), 413.3791 (C<sub>29</sub>H<sub>49</sub>O, 14), 357 (3), 331.2992 (C<sub>23</sub>H<sub>39</sub>O, 16), 315.2695 (C<sub>22</sub>H<sub>35</sub>O, 3), 287.2377 (C<sub>20</sub>H<sub>31</sub>O, 6), 269.2286 (C<sub>20</sub>H<sub>29</sub>, 2), 260.2134 (C<sub>18</sub>H<sub>28</sub>O, 13), 245.1901 (C<sub>17</sub>H<sub>25</sub>O, 100), 231.1743 (C<sub>16</sub>H<sub>23</sub>O, 23), 177.1641 (C<sub>13</sub>H<sub>21</sub>, 3), 177.1278 (C<sub>12</sub>H<sub>17</sub>O, 21), 98.1092 (C<sub>7</sub>H<sub>14</sub>, 77).

**Acknowledgment.** We thank F. Elaine DeJarnette and Phyllis Strong for technical assistance. We are indebted to our colleagues Lian Niang Li, Manfred Eggersdorfer, and Xian Li for the isolation of compounds 1a, 2b, and 3b, respectively, to James R. Lance, who mass cultured the *Orbulina universa* zooxanthellae and *Protoceraurum reticulatum* at Scripps Institution of Oceanography, La Jolla, CA, to Professor Robert K. Trench, University of Cali-

fornia at Santa Barbara, for an inoculum of these zooxanthellae, to Professor Francis T. Haxo, Scripps Institution of Oceanography, for an inoculum of *P. reticulatum*, to Professor George R. Pettit, Arizona State University, Tempe, AZ, for the extract of *Bugula neritina*, to Dr. J. C. Braekman, of the Free University of Brussels, for the sterol fraction of *Siphonogorgia* sp., to Dr. J. Baker, of the former Roche Research Institute of Marine Pharmacology, Dee Why, NSW, Australia, for a gift of *Stelletta conulosa*. The 360-MHz NMR spectra were recorded under the supervision of Dr. Lois Durham. Financial support from the National Institutes of Health (Grants No. AM-26546, No. GM-09840, and No. GM-28352) and the Wendt Foundation is gratefully acknowledged. Operation and maintenance of the Stanford 360-MHz NMR Facility were supported by NIH Grant No. RR-0711 and NSF Grant No. GP-23633. High-resolution mass spectra were recorded at the Midwest Center for Mass Spectrometry, University of Nebraska-Lincoln. This center is supported by grants from the National Science Foundation.

**Registry No.** 1a, 86708-32-9; 1b, 86708-33-0; 1c, 86708-34-1; 1d, 86708-35-2; 1e, 77617-71-1; 1f, 86708-36-3; 1i, 58670-63-6; 1j, 81445-03-6; 2a, 85505-68-6; 2b, 85505-67-5; 2c, 86708-37-4; 2d, 86708-38-5; 2g, 81520-53-8; 3b, 86708-39-6; 3g, 64783-84-2; 3j, 81445-04-7; 4f, 86708-40-9; 5a, 86708-41-0; 5b, 86708-42-1; 5c, 86708-43-2; 5d, 86708-44-3; 6a, 86708-45-4; 6b, 86709-22-0; 6c, 86708-46-5; 6d, 86708-47-6; 6f, 86708-48-7; 23(R),24(S)-dimethyl-5 $\alpha$ -cholestan-3 $\beta$ -ol *p*-bromobenzoate, 86668-14-6; fucosterol, 17605-67-3; cholesterol, 57-88-5; 24-methylenecholesterol, 474-63-5.

### Studies of Vitamin D Oxidation. 3. Dye-Sensitized Photooxidation of Vitamin D and Chemical Behavior of Vitamin D 6,19-Epidioxides

Sachiko Yamada, Keiko Nakayama, and Hiroaki Takayama\*

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

Akiko Itai and Yoichi Iitaka

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Received February 8, 1983

Dye-sensitized photooxidation of vitamin D and the chemical reactions of the resulting oxidation products have been studied in detail. Vitamin D undergoes 1,4-cycloaddition and ene-type reactions with singlet oxygen to yield two C(6) epimers of 6,19-epidioxvitamin D (3 and 4) as the major products (55–65% total isolated yields) and two C(6) epimers of the  $\Delta^{4,7,10(19)}$  6-hydroperoxide (5 and 6) as the minor products (15–25% total yields). The structures of the oxidation products are determined unambiguously by spectral data in combination with X-ray analysis. The chemical behavior of the endoperoxides 3 and 4 is examined in the reactions with basic reagents, Lewis and proton acids, transition-metal complexes, and reducing agents.

As a part of our studies<sup>1</sup> of the chemistry of the conjugated triene group of vitamin D, which is believed to play an important role in the biological activity of the vitamin,<sup>2</sup> we have been investigating the oxidation of the triene group. The oxidation is of interest not only from the chemical but also from the biological point of view, because

vitamin D apparently undergoes biological oxidation at the unsaturated part,<sup>3</sup> as unsaturated fatty acids do in the well-known biosynthesis of prostaglandins and leucotrienes.<sup>4</sup> Seeming to support this possibility is the recent

(1) (a) Yamada, S.; Nakayama, K.; Takayama, H. *Tetrahedron Lett.* 1978, 4895. (b) Yamada, S.; Takayama, H. *Chem. Lett.* 1979, 583. (c) Yamada, S.; Nakayama, K.; Takayama, H.; Itai, A.; Iitaka, Y. *Chem. Pharm. Bull.* 1979, 27, 1949. (d) Yamada, S.; Suzuki, T.; Takayama, H. *Tetrahedron Lett.* 1981, 22, 3085.

(2) DeLuca, H. F.; Schnoes, H. K. *Annu. Rev. Biochem.* 1976, 45, 631.

(3) Although many vitamin D metabolites were identified, the only one with an altered triene structure was 19-nor-7,8-epoxy-25-hydroxy-10-oxovitamin D<sub>2</sub>: DeLuca, H. F.; Schnoes, H. K.; Tanaka, Y. U.S. Patent 4229359, 1980.

(4) Samuelsson, B.; Granström, E.; Green, K.; Hamberg, M.; Hammarström, S. *Annu. Rev. Biochem.* 1975, 44, 889. Samuelsson, B.; Goldyne, M.; Granström, E.; Hamberg, M.; Hammarström, S.; Malmsten, C. *Ibid.* 1978, 47, 997. Samuelsson, B. *Pure Appl. Chem.* 1981, 53, 1203.

Table I. Photooxidation of Vitamin D<sub>3</sub> (1a) and D<sub>2</sub> (1b)

entry	substrate (mg)	solvent (mL)	sensitizer <sup>a</sup> (mg)	time, h	product isolated yield, %			
					3	4	5	6
1	1a (100)	EtOH (100)	RB (100)	3	26	29	11	7
2	1a (100)	CH <sub>2</sub> Cl <sub>2</sub> (100)	RB (100)	0.6	29	22	11	7
3	1a (100)	acetone (100)	RB (100)	1.5	28	28	11	7
4	1a (100)	EtOH-benzene (10:90)	RB (100)	0.6	34	30	12	9
5	1a (1000)	EtOH-benzene (20:180)	RB (200)	1.5	33	31	13	9
6	1a (100)	CH <sub>2</sub> Cl <sub>2</sub> (100)	TPP (5)	0.8	19	18	±	±
7	1a (100)	acetone (100)	TPP (5)	3	21	26	±	±
8	1b (100)	EtOH (100)	RB (100)	3	25	28	10	7
9	1b (1000)	EtOH-benzene (20:180)	RB (200)	2	31	34	14	10

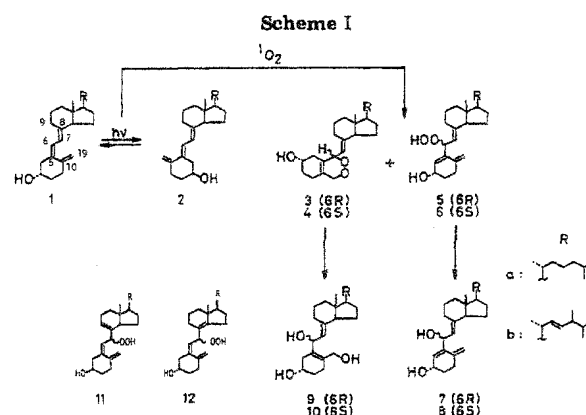
<sup>a</sup> The abbreviations used are as follows: RB, Rose Bengal; TPP, tetraphenylporphine.

discovery of the receptor protein of the active metabolite of vitamin D,<sup>5</sup> 1,25-dihydroxyvitamin D<sub>3</sub>, in human myeloid leukemia cells where lipoxygenase and singlet oxygen are known to be highly active.<sup>6</sup> The oxidation of vitamin D with singlet oxygen is of particular interest because the action of lipoxygenases resembles that of singlet oxygen and also because singlet oxygen is believed to be involved in some cases in peroxidation of lipids *in vivo*.<sup>7</sup> However, oxidation of vitamin D with singlet oxygen or with other oxidants has received little attention.<sup>8</sup>

Recently we reported the preliminary results of dye-sensitized photooxidation of vitamin D and the successful isolation of the vitamin D-singlet oxygen adducts 3 and 4.<sup>1a</sup> It has also been found<sup>9</sup> that the vitamin D<sub>3</sub>-singlet oxygen adducts 3a and 4a are significantly active in intestinal calcium transport and in the elevation of serum calcium and phosphophate levels in vitamin D deficient animals. Now we studied the photooxidation of vitamin D in more detail and found that vitamin D undergoes an ene-type reaction with singlet oxygen in addition to the 1,4-cycloaddition reaction. We have also studied the chemical reactivity of the vitamin D 6,19-epidioxides 3 and 4 and found interesting reactions. Here we report the results in detail.

### Results and Discussion

**Photooxidation.** Photooxidation of vitamin D<sub>3</sub> and D<sub>2</sub> (1a and 1b) was examined in various solvents by using Rose Bengal (RB) and tetraphenylporphine (TPP) as sensitizers. The oxidation was terminated when almost all of the starting vitamin was consumed. The results are summarized in Table I. Vitamin D underwent both 1,4-cycloaddition and ene-type reactions with singlet oxygen at the conjugated triene part. 1,4-Cycloaddition at the *s*-cis diene part was the major oxidation pathway, giving rise to the two isomeric endoperoxides 3 and 4 in 55–65% total isolated yields (Scheme I). When RB was used as the sensitizer, hydroperoxides 5 and 6 were obtained as a result of the ene-type reaction of singlet oxygen. The ene reaction occurred regioselectively at the trisubstituted 5(6)



double bond, abstracting the allylic proton at C(4) to yield the two C(6) epimers of the  $\Delta^{4,7,10(19)}$  6-hydroperoxide, 5 and 6, in 15–25% total yields. These hydroperoxides could not be isolated from the oxidation products by using TPP as the sensitizer, but the formation was detected on TLC among a number of by products with similar polarity. The rate of photooxidation depended on the reaction conditions, especially the kind of solvent used which may affect the lifetime of the singlet oxygen. However, the ratios of the two types of products were little affected by the nature of the solvent. With the same conditions under an atmosphere of oxygen-free inert gas, *cis*-*trans* isomerization of the 5(6) double bond occurred in the starting vitamin D, yielding an equilibrium mixture of 1 and the 5,6-*trans* isomer (2), as reported.<sup>10</sup> Since the isomerization is faster than the oxidation, it is clear that the oxidation products are derived from both vitamin D isomers 1 and 2 in a photoequilibrium state.

**Characterization of the Photooxidation Products.** The structures of the major products 3 and 4 were determined by spectral analysis to be the C(6) epimers of the dioxygen adducts of the starting vitamin D at the *s*-*cis* diene part (Table II). The mass spectra and elemental analysis corroborate the molecular composition. The UV spectra show no absorption maximum above 220 nm, indicating the absence of a conjugated double bond. The <sup>1</sup>H NMR spectra exhibit the resonances of the C(19) protons as an AB quartet or a broad singlet at  $\delta$  4.0–4.7 and the resonances of the protons at C(6) and C(7) as a pair of doublets at  $\delta$  4.7–5.4. The <sup>13</sup>C NMR spectra indicate four *sp*<sup>2</sup> carbons, besides those of the side-chain

(5) Abe, E.; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* 1981, 78, 4990. Miyaura, C.; Abe, E.; Kuribayashi, T.; Tanaka, H.; Konno, K.; Nishii, Y.; Suda, T. *Biochem. Biophys. Res. Commun.* 1981, 102, 937. Tanaka, H.; Abe, E.; Miyaura, C.; Kuribayashi, T.; Konno, K.; Nishii, Y.; Suda, T. *Biochem. J.* 1982, 204, 715.

(6) Krinsky, N. I. *Science (Washington, D.C.)* 1974, 186, 363.  
(7) Pederson, T. C.; Aust, S. D. *Biochim. Biophys. Acta* 1975, 385, 232. Kellogg, E. W.; Fridovich, I. *J. Biol. Chem.* 1975, 250, 8812. Pederson, T. C.; Aust, S. D. *Biochem. Biophys. Res. Commun.* 1973, 52, 1071.  
(8) Bland, J.; Craney, B. *Tetrahedron Lett.* 1974, 4041.

(9) Moriuchi, S.; Tsuruki, F.; Ohtawara, Y.; Hosoya, N.; Yamada, S.; Nakayama, K.; Takayama, H. *J. Nutr. Sci. Vitaminol.* 1979, 25, 455.

(10) Gielen, J. W. J.; Koolstra, R. B.; Jacobs, H. J. C.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* 1980, 99, 306.

Table II. Spectral Data of Vitamin D<sub>2</sub> Endoperoxides 3 and 4

compd	<sup>1</sup> H NMR, <sup>a</sup> $\delta$ (multiplicity, <i>J</i> in Hz)			<sup>13</sup> C NMR, <sup>a</sup> $\delta$ (multiplicity)							CD, <sup>b</sup> nm ( $\Delta\epsilon$ )
	H-6 and H-7		H-19	C-5 and C-10		C-7	C-8	C-3	C-6	C-19	
3a	4.88 (d, 10)	5.17 (d, 10)	4.36 (br s)	125.7 (s)	125.7 (s)	115.6 (d)	148.1 (s)	65.9 (d)	76.9 (d)	72.2 (t)	207 (-23.3)
4a	4.76 (d, 9)	5.23 (d, 9)	4.17 (d, 16), 4.60 (d, 16)	126.8 (s)	125.7 (s)	114.5 (d)	149.2 (s)	67.2 (d)	76.8 (d)	72.0 (t)	215 (+6.5)
3b	4.85 (d, 9)	5.12 (d, 9)	4.36 (br s)	125.8 (s)	125.8 (s)	115.5 (d)	148.3 (s)	66.1 (d)	76.9 (d)	72.2 (t)	210 (-17.7)
4b	4.73 (d, 10)	5.18 (d, 10)	4.08 (d, 16), 4.48 (d, 16)	126.7 (s)	125.8 (s)	114.6 (d)	149.3 (s)	67.5 (d)	77.0 (d)	72.1 (t)	211 (+11.3)
3c	4.88 (d, 10)	5.32 (d, 10)	4.30 (d, 15), 4.56 (d, 15)	125.9 (s)	125.4 (s)	114.9 (d)	148.9 (s)	69.0 (d)	76.7 (d)	72.1 (t)	211 (-11.5), 227 (+5.7)
4c	4.78 (d, 10)	5.30 (d, 10)	4.21 (d, 16), 4.63 (d, 16)	126.3 (s)	125.9 (s)	114.3 (d)	149.6 (s)	70.6 (d)	76.8 (d)	72.0 (t)	206 (+7.7), 219 (+5.8)

<sup>a</sup> CDCl<sub>3</sub> as the solvent. <sup>b</sup> Hexane as the solvent.

Table III. Spectral Data of Hydroperoxides and Related Compounds

compd	<sup>1</sup> H NMR, <sup>a</sup> (multiplicity, <i>J</i> in Hz)						MS, <i>m/e</i>	UV max, <sup>b</sup> nm
	H <sub>3</sub> -18	H-19		H-6 and H-7		H-4		
5a	0.52 (s)	4.96 (br s)	5.13 (br s)	5.00 (d, 9)	5.54 (d, 9)	6.02 (m)	416, 400, 398, 382	232
6a	0.60 (s)	4.96 (br s)	5.12 (br s)	5.00 (d, 9)	5.53 (d, 9)	6.04 (m)	416, 400, 398, 382	232.5
5b	0.52 (s)	4.97 (br s)	5.0-5.4 <sup>c</sup>		5.54 (d, 8)	6.04 (m)	428, 412, 410, 394	235
6b	0.61 (s)	4.95 (br s)	4.9-5.3 <sup>c</sup>		5.50 (d, 8)	6.04 (m)	428, 412, 410, 394	234
7a	0.50 (s)	4.94 (br s)	5.06 (br s)	5.10 (d, 8)	5.30 (d, 8)	6.12 (m)	400, 382, 364	234.5
8a	0.60 (s)	4.90 (br s)	5.00 (br s)	5.04 (d, 8)	5.24 (d, 8)	6.05 (m)	400, 382, 364	234.5

<sup>a</sup> CDCl<sub>3</sub> as the solvent. <sup>b</sup> 95% EtOH as the solvent. <sup>c</sup> The signals are superimposed by the signals of H-22 and H-23.

carbons in the vitamin D<sub>2</sub> derivatives 3b and 4b, and three sp<sup>3</sup> carbons adjacent to an oxygen atom. The stereochemistry of the peroxides 3 and 4 at C(6) was determined by X-ray analysis in collaboration with the CD spectra. Although the pair of epimeric peroxides 3 and 4 show quite similar properties in the spectra described so far, they show contrasting behavior in their CD spectra (Table II). While the epimer 3 shows a negative Cotton effect around 210 nm, due to the homoconjugated double bond, the other isomer, 4, shows a positive Cotton effect at the same wavelength region. This indicates that the configuration at C(6) has a pronounced effect on the sign of the Cotton effect and that the latter is related to the chirality of these homoconjugated systems. The stereochemistry of crystalline benzoate 3c of vitamin D<sub>2</sub> endoperoxide 3b was determined by single-crystal X-ray analysis.<sup>11</sup> The ORTEP drawing of the structure of 3c (Figure 1) shows that 3c has the 6*R* configuration. Thus, it was established that around 210 nm the isomers 3b and 3c which show a negative Cotton effect have the 6*R* configuration and that the other isomers 4b and 4c which show a positive Cotton effect have the 6*S* configuration. The stereochemistry of the vitamin D<sub>3</sub> endoperoxides 3a and 4a at C(6) was deduced by comparing their CD spectra with those of the vitamin D<sub>2</sub> derivatives 3b and 4b.

The minor products 5 and 6 were determined by spectral analysis and specific chemical reactions to have the

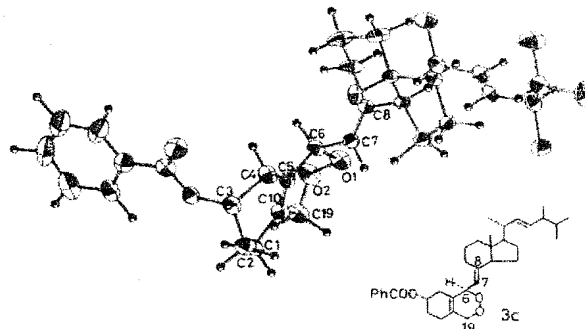


Figure 1. ORTEP drawing of 3c.

structures of the C(6) epimers of the  $\Delta^{4,7,10(19)}$  6-hydroperoxide (Table III). The mass spectra show a weak molecular ion, indicating the incorporation of two oxygen atoms in the molecule. The fragment ions characteristic for hydroperoxides<sup>12</sup> appear at  $M^+ - O$ ,  $M^+ - H_2O$ , and  $M^+ - H_2O_2$ . By Woodward's rule,<sup>13</sup> the absorption maximum in the UV spectra is in good agreement with that calculated for the chromophore of the assigned structures. The <sup>1</sup>H NMR spectra exhibit the resonance of the 18-methyl group in the normal region, indicating the absence of an 8(9) or 8(14) double bond.<sup>14</sup> This excludes the alternative structures 11 and 12. The <sup>1</sup>H NMR spectra also exhibit

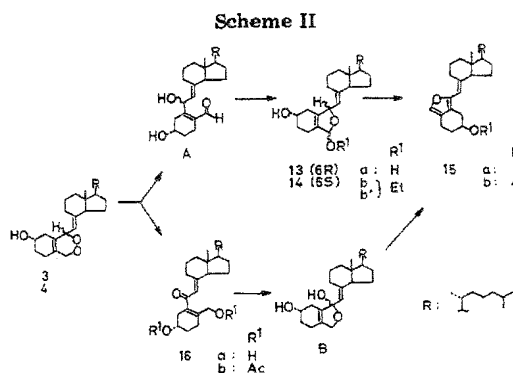
(11) The crystals were monoclinic *P*2<sub>1</sub> with cell dimensions of *a* = 16.753 Å, *b* = 7.446 Å, *c* = 13.572 Å, and  $\beta$  = 111.4°. Intensities were measured on a Philips PW1100 four-circle diffractometer by using Cu K $\alpha$  radiation monochromated by a graphite plate, and 3144 independent data were used for the analysis. The structure was elucidated by the direct method with the program MULTAN (Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr. Sect. A* 1971, A27, 368). Positional and thermal parameters were refined by the least-squares method to an *R* value of 0.092.

(12) Lear, J. E.; Smith, L. L. *J. Org. Chem.* 1971, 36, 1007. Feinberg, A. M.; Nakanishi, K.; Barciszewski, J.; Rafalski, A. J.; Augustyniak, H.; Wiewiorowski, M. *J. Am. Chem. Soc.* 1974, 96, 7797.

(13) Woodward, R. B. *J. Am. Chem. Soc.* 1941, 63, 1123; 1942, 64, 72; 1942, 64, 76.

(14) The chemical shifts for the 18-methyl group are indicative of the position of the double bond at the C-ring; the resonances are at  $\delta$  ca. 0.55, 0.70, and 0.90 for the  $\Delta^7$ ,  $\Delta^{8(9)}$ , and  $\Delta^{8(14)}$  compounds, respectively.

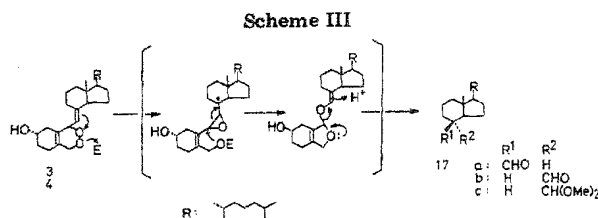




the resonances of the intact exocyclic methylene group at C(19) as a pair of broad singlets, the resonances of the protons at C(6) and C(7) as a pair of doublets, the resonance of a proton at C(4) in an olefinic proton region, and the resonance of H(3) which is shifted to lower field, indicating the presence of the 4(5) double bond. Reduction of the hydroperoxides 5a and 6a with triphenylphosphine yielded the diols 7a and 8a, the structure of which was verified by the spectral data (Table III). The stereochemistry of the hydroperoxides 5 and 6 and the diols 7 and 8 at C(6) was assigned by comparing their  $^1\text{H}$  NMR spectra with those of the structurally closely related (6*R*)- and (6*S*)-triols 9 and 10 obtained from (6*R*)- and (6*S*)-vitamin D<sub>3</sub> endoperoxides 3a and 4a by reduction with  $\text{LiAlH}_4$  (see below). In these 6-hydroxy and 6-hydroperoxy derivatives the chemical shift of the 18-methyl group reflects the stereochemistry at C(6); the resonances of 6*R* isomers appear at a field higher than that of the 6*S* isomers by about 0.1 ppm.

**Reactions of Vitamin D Endoperoxides.** The endoperoxides 3 and 4 are thermally rather stable and are recovered unchanged even after refluxing in xylene for several hours, if the substrate and the solvent used are purified rigorously. However, they are sensitive to bases, acids, and transition-metal complexes.

**Reaction with Basic Reagents.** On treatment with 5% KOH in methanol at room temperature 3a was converted within 30 min to furan 15a (18%) and hemiacetal 13a (69%), as a mixture of the C(19) epimers (Scheme II). The structure of 15a was based on the spectral data. The mass spectrum showed a molecular ion at  $m/e$  398. The UV spectrum showed characteristic absorption maxima due to the vinyl furan chromophore at 273 nm ( $\log \epsilon$  4.20), 283 (4.26), 295.5 (4.12). The  $^1\text{H}$  NMR spectrum exhibited an aromatic proton on the furan ring at  $\delta$  7.15 as a singlet and a vinyl proton at C(7) at  $\delta$  5.64 as a singlet. The structure of the hemiacetal 13a was determined on the basis of the spectral data [mass spectrum,  $m/e$  416 ( $\text{M}^+$ );  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.61 (3 H, s, H-18), 3.56–5.90 (6 H, complex signal); IR ( $\text{CHCl}_3$ ) 3610, 3420, 2940  $\text{cm}^{-1}$ ] and the following characteristics: (i) compound 13a was labile and dehydrated to give the furan 15a even on standing at room temperature in  $\text{CDCl}_3$ , probably due to the action of a trace of acid in the  $\text{CDCl}_3$ ; (ii) on standing in ethanol 13a was transformed into two isomeric acetals, less polar 13b and more polar 13b', both of which in turn were converted quantitatively to the furan (15a) by refluxing in xylene; (iii) the spectral properties of the acetals 13b and 13b' were in good agreement with the assigned structure. The mass spectra of 13b and 13b' showed a molecular ion at  $m/e$  444. The  $^1\text{H}$  NMR spectra of 13b and 13b' showed the resonances of the ethoxy group [13b:  $\delta$  1.11 (3 H, t,  $J = 7$  Hz), 3.46 (2 H, m). 13b':  $\delta$  1.12 (3



H, t,  $J = 7$  Hz), 3.51 (2 H, m)], the resonances of the protons at C(6) and C(7) as a pair of doublets [13b:  $\delta$  4.60 (1 H, d,  $J = 9$  Hz), 5.36 (1 H, d,  $J = 9$  Hz). 13b':  $\delta$  4.76 (1 H, d,  $J = 9$  Hz), 5.17 (1 H, d,  $J = 9$  Hz)], and the resonance of the acetal proton at C(19) as a singlet (13b,  $\delta$  5.48; 13b'  $\delta$  5.39). In this reaction, formation of the regioisomer of the hemiacetal 13a, hemiketal B, was not observed. The hemiketal B formed first probably was dehydrated to the furan 15a under the reaction conditions, since the hemiacetal 13a did not afford the furan 15a under the same reaction conditions. Keto-alcohol 16a, a precursor of the hemiketal B, was trapped as acetate 16b when 3a was treated with acetic anhydride–pyridine (1:1, 50 °C); 3a yielded 15b (70%) and the ketone 16b (18%).<sup>15</sup> The transformation of the endoperoxide 3a to the furan 15a and the hemiacetal 13a was also effected by the other basic reagents such as triethylamine (benzene, 80 °C), and  $\text{CsF}$  (DMF). The two C(6) epimers 3a and 4a showed little difference in their chemical behavior in the reactions examined in the present studies. So only the reactions of the 6*R* isomer 3a will be discussed here as representative of both; the reactions of the 6*S* isomer are described in the Experimental Section.

**Reaction with Acidic Reagents.** The reactions of the vitamin D endoperoxides 3 and 4 with acidic reagents, in which cleavage of the bond between C(6) and C(7) occurs, are rather unusual. Treatment of 3a with Lewis acids such as  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (benzene, room temperature) and  $\text{ZnCl}_2$  (xylene, 100 °C) produced aldehyde 17a in good yields (~85%). The structure of 17a was deduced on the basis of the spectral data and some chemical reactions. The mass spectrum exhibited a molecular ion at  $m/e$  278. The  $^1\text{H}$  NMR spectrum exhibited an aldehyde proton at  $\delta$  9.99 as a singlet. The IR spectrum showed an absorption of the carbonyl group at 1710  $\text{cm}^{-1}$ . The  $\beta$  configuration of the formyl group was revealed by the fact that 17a was converted quantitatively to the thermodynamically more stable isomer 17b [ $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.53 (1 H, d,  $J = 3$  Hz, CHO); IR ( $\text{CHCl}_3$ ) 1720  $\text{cm}^{-1}$ ] under equilibrium conditions ( $\text{EtONa}$ ,  $\text{EtOH}$ ). Proton acids effected a similar transformation. Thus, by treatment with  $\text{HCl}$  ( $\text{MeOH}$ , 0 °C), 3a afforded acetal 17c (38%). The structure of 17c was based on the spectral data [mass spectrum,  $m/e$  324 ( $\text{M}^+$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.38 (3 H, s, MeO), 3.40 (3 H, s, MeO), 4.06 (1 H, d,  $J = 4$  Hz, acetal CH)] and on the fact that 17c was transformed quantitatively into the aldehyde 17b by treatment with acidic aqueous acetone. The configuration of the acetal group was considered to be  $\alpha$  (equatorial), since it was produced under thermodynamic conditions. The mechanism shown in Scheme III is suggested for the reactions in acidic conditions.<sup>16</sup> The ste-

(15) The acetyl derivative of the hydroxy aldehyde A was not detected in the reaction of 3a (or 4a) with acetic anhydride–pyridine. The hydroxy aldehyde A probably underwent cyclization between the hydroxyl group at C(6) and the 19-formyl group and subsequent dehydration to yield the furan 15, before it was acetylated at the sterically hindered 6-hydroxyl group.

(16) Products arising from the A-ring part could not be isolated from the reaction.

reoselective formation of the thermodynamically less stable  $\beta$ -formyl derivative 17a in the Lewis acid catalyzed reactions can be rationalized by assuming a kinetic protonation from the less hindered  $\alpha$  side of the molecule.

**Reactions with Transition-Metal Complexes.** The reactions of endoperoxides catalyzed by transition-metal complexes have been investigated recently in connection with biological transformations.<sup>17</sup> The catalytic reaction of vitamin D endoperoxides 3a and 4a was examined by using two types of transition-metal complexes: a Co(II) complex which is capable of inducing the reaction via a one-electron redox process, and a Pd(0) complex which can act as a two-electron transfer reagent. Cobalt-tetraphenylporphine (CoTPP) complex has been known to isomerize sterically compressed bicyclic endoperoxides to diepoxides.<sup>17a</sup> In the particular endoperoxide 3a this reagent was highly effective in transforming the peroxide exclusively to the hemiacetal 13a ( $\text{CH}_2\text{Cl}_2$ , -20 to -10 °C; 75% yield). The high regioselectivity observed in this reaction is probably due to the selective formation of a metal complex at the less hindered oxygen atom bound to C(19). Pd(0) complex<sup>17b,c</sup> caused a similar transformation; on treatment with Pd( $\text{Ph}_3\text{P}$ )<sub>4</sub> (benzene, reflux), 3a afforded 13a (16%) and 15a (47%). These reactions probably proceed via the hydroxy enal (A) or the hydroxy enone 16a as an intermediate, as in the reaction with basic reagents (Scheme II).

**Reductions.** The endoperoxides 3 and 4 were stable for mild reducing agents such as thiourea,<sup>18</sup>  $\text{NaBH}_4$ , and diimide<sup>19</sup> and for catalytic hydrogenation. The peroxide 3a was readily reduced by  $\text{LiAlH}_4$  ( $\text{Et}_2\text{O}$ , room temperature) to triol 9 in good yield (70%). The triol 9 was labile and decomposed even with chromatography on a silica gel column. Purification of the triol 9 was achieved only by using gel chromatography (Sephadex LH-20).

### Experimental Section

**General Methods.** Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Hitachi 215 spectrophotometer. Proton magnetic resonance (<sup>1</sup>H NMR) spectra were recorded with a Varian XL-100 spectrometer with tetramethylsilane as an internal standard. Carbon magnetic resonance (<sup>13</sup>C NMR) spectra were recorded with a Varian XL-100 spectrometer at 25.16 MHz with tetramethylsilane as an internal reference. Mass spectra (MS) were recorded with a JEOL JMS-D300 GS/MS instrument with an interfaced computer. Ultraviolet (UV) spectra were recorded with a Union Giken SM-401 spectrophotometer. Circular dichroism (CD) spectra were recorded with a JASCO J-20A spectropolarimeter.

**Photooxidation of Vitamin D Derivatives (1a and 1b).** A solution of vitamin D (1) and a sensitizer was placed in an immersion vessel, purged with oxygen, and irradiated with a water-cooled 200-W halogen lamp (Ushio JCV 100-200GS). Oxygen was kept bubbling through the solution during the irradiation and the outside of the vessel was cooled with an ice. The reaction was monitored by TLC and was terminated when almost all of the starting material was consumed. The solvent was evaporated, and the residue was dissolved in ethyl acetate, washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel with ethyl acetate-benzene (4:96) as the eluent to afford (6S)-epidioxide 4, (6R)-epidioxide 3, and a mixture of the two epimeric hydroperoxides

5 and 6, in this order. The two isomeric hydroperoxides 5 and 6 were separated by using HPLC (Lichrosorb Si-60, 0.4 × 25 cm, *i*-PrOH-hexane 7:93) to give less polar 6 and more polar 5. The results are summarized in Table I. 3a: high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_3$  requires  $m/e$  416.3290, found  $m/e$  416.3306; MS,  $m/e$  416 ( $\text{M}^+$ ), 398, 285; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.56 (3 H, s, H-18), 4.07 (1 H, m, H-3). 4a: high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_3$  requires  $m/e$  416.3290, found  $m/e$  416.3270; MS,  $m/e$  416 ( $\text{M}^+$ ), 398, 285; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.57 (3 H, s, H-18), 3.93 (1 H, m, H-3). 5a: high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_3$  requires  $m/e$  416.3290, found  $m/e$  416.3276; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.42 (1 H, m, H-3), 7.86 (1 H, s, OOH,  $\text{D}_2\text{O}$  exchangeable); IR ( $\text{CHCl}_3$ ) 3350, 2950  $\text{cm}^{-1}$ . 6a: high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_3$  requires  $m/e$  416.3290, found  $m/e$  416.3280; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.43 (1 H, m, H-3), 8.08 (1 H, s, OOH,  $\text{D}_2\text{O}$  exchangeable); IR ( $\text{CHCl}_3$ ) 3350, 2950  $\text{cm}^{-1}$ . 3b: high-resolution MS,  $\text{C}_{28}\text{H}_{44}\text{O}_3$  requires  $m/e$  428.3290, found  $m/e$  428.3289; MS,  $m/e$  428 ( $\text{M}^+$ ), 410, 285; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.55 (3 H, s, H-18), 4.07 (1 H, m, H-3), 5.12 (2 H, m, H-22 and H-23). 4b: high-resolution MS,  $\text{C}_{28}\text{H}_{44}\text{O}_3$  requires  $m/e$  428.3290, found  $m/e$  428.3295; MS,  $m/e$  428 ( $\text{M}^+$ ), 410, 285; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.54 (3 H, s, H-18), 3.78 (1 H, m, H-3), 5.12 (2 H, m, H-22 and H-23). 5b: high-resolution MS,  $\text{C}_{28}\text{H}_{44}\text{O}_3$  requires  $m/e$  428.3290, found  $m/e$  428.3287; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.43 (1 H, m, H-3), 7.96 (1 H, s, OOH,  $\text{D}_2\text{O}$  exchangeable); IR ( $\text{CHCl}_3$ ) 3350, 2950  $\text{cm}^{-1}$ . 6b: high-resolution MS,  $\text{C}_{28}\text{H}_{44}\text{O}_3$  requires  $m/e$  428.3290, found  $m/e$  428.3289; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.42 (1 H, m, H-3), 8.28 (1 H, s, OOH,  $\text{D}_2\text{O}$  exchangeable); IR ( $\text{CHCl}_3$ ) 3350, 2950  $\text{cm}^{-1}$ .

(6R)-6,19-Epidioxy-9,10-secoergosta-5(10),7,22-trien-3 $\beta$ -yl Benzoate (3c). Benzoyl chloride (36  $\mu\text{L}$ , 0.31 mmol) was added to a solution of 3b (110 mg, 0.26 mmol) in pyridine (0.2 mL) at 0 °C. After 30 min, ice chips were added, and the mixture was extracted with ethyl acetate. The extract was washed with aqueous  $\text{NaHCO}_3$  and water, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel (6 g) with ethyl acetate-hexane (2:98) as the eluent to yield 3c: 115 mg (84%); mp 132–133 °C (from hexane); MS,  $m/e$  532 ( $\text{M}^+$ ), 514, 392, 267; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.59 (3 H, s, H-18), 0.82, 0.84, 0.92, and 1.02 (each 3 H, d,  $J$  = 7 Hz, H-21, H-26, H-27, and H-28), 4.3 (1 H, d,  $J$  = 15 Hz, H-19), 4.56 (1 H, d,  $J$  = 15 Hz, H-19), 4.88 (1 H, d,  $J$  = 10 Hz, H-6 or H-7), 5.18 (2 H, m, H-22 and H-23), 5.32 (1 H, d,  $J$  = 10 Hz, H-7 or H-6), 5.4 (1 H, m, H-3), 7.3–7.6 (3 H, m, H-Ar), 8.01 (2 H, dd,  $J$  = 8, 2 Hz, H-Ar); IR (KBr) 2960, 1710, 1275, 710  $\text{cm}^{-1}$ ; UV max (95% EtOH) 227 nm ( $\log \epsilon$  4.19);  $[\alpha]_D^{25} +77.5^\circ$  (c 0.69,  $\text{CHCl}_3$ ). Anal. Calcd for  $\text{C}_{35}\text{H}_{48}\text{O}_4$ : C, 78.91; H, 9.08. Found: C, 78.62; H, 9.01.

(6S)-6,19-Epidioxy-9,10-secoergosta-5(10),7,22-trien-3 $\beta$ -yl Benzoate (4c). In a similar manner, 4b (100 mg, 0.23 mmol) was converted to the benzoate 4c: 98 mg (79%) mp 126–127 °C (from hexane); MS,  $m/e$  532 ( $\text{M}^+$ ), 514, 392, 267; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  0.59 (3 H, s, H-18), 0.83, 0.84, 0.92, and 1.03 (each 3 H, d,  $J$  = 7 Hz, H-21, H-26, H-27, and H-28), 4.21 (1 H, d,  $J$  = 16 Hz, H-19), 4.63 (1 H, d,  $J$  = 16 Hz, H-19), 4.78 (1 H, d,  $J$  = 10 Hz, H-6 and H-7), 5.18 (2 H, m, H-22 and H-23), 5.25 (1 H, m, H-3), 5.30 (1 H, d,  $J$  = 10 Hz, H-7 or H-6), 7.3–7.6 (3 H, m, H-Ar), 8.01 (2 H, dd,  $J$  = 8, 2 Hz, H-Ar); IR (KBr) 2960, 1720, 1275, 710  $\text{cm}^{-1}$ ; UV max (95% EtOH) 229 nm ( $\log \epsilon$  4.18);  $[\alpha]_D^{25} -6.1^\circ$  (c 0.54,  $\text{CHCl}_3$ ). Anal. Calcd for  $\text{C}_{35}\text{H}_{48}\text{O}_4$ : C, 78.91; H, 9.08. Found: C, 79.06; H, 9.13.

(6R)-9,10-Secocholesta-4,7,10(19)-triene-3 $\beta$ ,6-diol (7a). A solution of 5a (10 mg,  $2.4 \times 10^{-2}$  mmol) and triphenylphosphine (6.5 mg,  $2.5 \times 10^{-2}$  mmol) in benzene (600  $\mu\text{L}$ ) was stirred at room temperature for 20 min. The solvent was evaporated, and the residue was chromatographed on silica gel (3 g) with ethyl acetate-hexane (1:1) as the eluent to afford diol 7a: 8.3 mg (86%); high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_2$  requires  $m/e$  400.3341, found  $m/e$  400.3339; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.40 (1 H, m, H-3); IR ( $\text{CHCl}_3$ ) 3610, 3400, 2950  $\text{cm}^{-1}$ .

(6S)-9,10-Secocholesta-4,7,10(19)-triene-3 $\beta$ ,6-diol (8a). In a similar manner, 6a (8 mg,  $1.9 \times 10^{-2}$  mmol) was treated with triphenyl phosphine (5.2 mg,  $2 \times 10^{-2}$  mmol) to afford 8a: 6 mg (78%); high-resolution MS,  $\text{C}_{27}\text{H}_{44}\text{O}_2$  requires  $m/e$  400.3341, found  $m/e$  400.3324; <sup>1</sup>H NMR ( $\text{CDCl}_3$ )  $\delta$  4.38 (1 H, m, H-3); IR ( $\text{CHCl}_3$ ) 3600, 3410, 2950  $\text{cm}^{-1}$ .

**Reaction of Endoperoxides 3a and 4a with Methanolic KOH.** Peroxide 3a (55 mg,  $1.3 \times 10^{-1}$  mmol) was dissolved in 5% methanolic KOH (3 mL), and the solution was stored at room

(17) (a) Boyd, J. D.; Foote, C. S.; Imagawa, D. K. *J. Am. Chem. Soc.* 1980, 102, 3641. (b) Suzuki, M.; Noyori, R.; Hamanaka, N. *Ibid.* 1981, 103, 5606. (c) Suzuki, M.; Oda, Y.; Noyori, R. *Tetrahedron Lett.* 1981, 22, 4413.

(18) Kaneko, C.; Sugimoto, A.; Tanaka, S. *Synthesis* 1974, 876.

(19) Adam, W.; Eggelte, H. J. *Angew. Chem., Int. Ed. Engl.* 1977, 16, 713; *J. Org. Chem.* 1977, 42, 3978. Adam, W.; Bloodworth, A. J.; Eggelte, H. J.; Loveitt, M. E. *Angew. Chem., Int. Ed. Engl.* 1978, 17, 209.



temperature for 30 min. The mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. Chromatography of the residue on silica gel (5 g) with ethyl acetate-hexane (2:3) as the eluent gave furan 15a (9.5 mg, 18%). Further elution with ethyl acetate-hexane (8:2) afforded hemiacetal 13a: 38 mg (69%); MS,  $m/e$  416 ( $M^+$ ), 398, 285, 191, 190, 151;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.61 (3 H, s, H-18) 3.56–5.90 (6 H, complex); IR ( $\text{CHCl}_3$ ) 3610, 3420, 2940  $\text{cm}^{-1}$ . For 15a: MS,  $m/e$  398 ( $M^+$ ), 285, 191, 190, 151;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.62 (3 H, s, H-18), 3.99 (1 H, m, H-3), 5.64 (1 H, s, H-7), 7.15 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 2940  $\text{cm}^{-1}$ ; UV max (95% EtOH) 273.5 nm (log  $\epsilon$  4.20), 283 (4.26), 295.5 (4.12).

In a similar manner, 4a (100 mg,  $2.4 \times 10^{-1}$  mmol) was transformed into 15a (18 mg, 19%) and 14a (71 mg, 71%) on treatment with 5% methanolic KOH (5 mL). 14a: MS,  $m/e$  416 ( $M^+$ ), 398, 285, 191, 190, 151;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.60 (3 H, s, H-18), 3.50–5.93 (6 H, complex).

Hemiacetal 13a (30 mg) was dissolved in 5% methanolic KOH (2 mL), and the solution was stored at room temperature for 30 min. After a similar workup and chromatographic purification, 27 mg (90%) of 13a was recovered unchanged.

**(6R)-6,19-Epoxy-19-ethoxy-9,10-secocholesta-5(10),7-dien-3 $\beta$ -ols (13b and 13b').** Hemiacetal 13a (40 mg,  $9.6 \times 10^{-2}$  mmol) was dissolved in EtOH, and the solution was stored at 3 °C for 3 days and then at room temperature for 4 days. After evaporation of the solvent, the residue was purified by using HPLC ( $\mu$ -Porasil, 0.79  $\times$  30 cm, 5:95 *i*-PrOH-hexane) to afford less polar 13b (22 mg, 52%) and more polar 13b' (15 mg, 35%). 13b: MS,  $m/e$  444 ( $M^+$ ), 398, 285, 151;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.60 (3 H, s, H-18), 1.11 (3 H, t,  $J$  = 7 Hz, EtO), 3.46 (2 H, m, EtO), 3.68 (1 H, d,  $J$  = 4.5 Hz, OH), 3.88 (1 H, m, H-3), 4.60 (1 H, d,  $J$  = 9 Hz, H-6 or H-7), 5.36 (1 H, d,  $J$  = 9 Hz, H-7 or H-6), 5.48 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 3400, 2955  $\text{cm}^{-1}$ . 13b': MS,  $m/e$  444 ( $M^+$ ), 398, 285, 151;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.60 (3 H, s, H-18), 1.12 (3 H, t,  $J$  = 7 Hz, EtO), 3.51 (2 H, m, EtO), 3.70 (1 H, d,  $J$  = 4.5 Hz, OH), 3.90 (1 H, m, H-3), 4.76 (1 H, d,  $J$  = 9 Hz, H-6 or H-7), 5.17 (1 H, d,  $J$  = 9 Hz, H-7 or H-6), 5.39 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 3400, 2955  $\text{cm}^{-1}$ .

**(6S)-6,19-Epoxy-19-ethoxy-9,10-secocholesta-5(10),7-dien-3 $\beta$ -ols (14b and 14b').** In a similar manner, hemiacetal 14a (40 mg) was converted to acetals 14b (15 mg, 35%) and 14b' (22 mg, 52%) on standing in ethanol. 14b: MS,  $m/e$  444 ( $M^+$ ), 398, 285, 151;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.60 (3 H, s, H-18), 1.25 (3 H, t,  $J$  = 7 Hz, EtO), 3.66 (2 H, m, EtO), 4.03 (1 H, m, H-3), 4.92 (1 H, d,  $J$  = 8 Hz, H-6 or H-7), 5.33 (1 H, d,  $J$  = 8 Hz, H-7 or H-6), 5.60 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 3440, 2950  $\text{cm}^{-1}$ . 14b': MS,  $m/e$  444 ( $M^+$ ), 398, 285, 151;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.60 (3 H, s, H-18), 1.24 (3 H, t,  $J$  = 7 Hz, EtO), 3.68 (2 H, m, EtO), 4.10 (1 H, m, H-3), 4.74 (1 H, d,  $J$  = 8 Hz, H-6 or H-7), 5.59 (1 H, d,  $J$  = 8 Hz, H-7 or H-6), 5.66 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 3450, 2950  $\text{cm}^{-1}$ .

**6,19-Epoxy-9,10-secocholesta-5,7,10(19)-trien-3 $\beta$ -ol (15a).** (A) From Hemiacetal 13a. A solution of 13a (34 mg,  $8.2 \times 10^{-2}$  mmol) in  $\text{CDCl}_3$  (500  $\mu\text{L}$ ) was kept at room temperature for 2 h. The solvent was evaporated, and the residue was chromatographed on silica gel (5 g) with ethyl acetate-hexane (1:1) as the eluent to yield furan 15a (31 mg, 95%).

(B) From Acetals 13b and 13b'. A solution of acetal 13b (2 mg) in xylene (1 mL) was refluxed for 1 h. The solvent was evaporated, and the residue was purified on TLC (silica gel; ethyl acetate-hexane, 15:75) to give 15a (1.8 mg, 94%).

In a similar manner, 13b' (3 mg) was converted to 15a (2.5 mg, quantitative).

**Reaction of Endoperoxides 3a and 4a with Acetic Anhydride-Pyridine.** Acetic anhydride (2 mL) was added to a solution of 3a (95 mg) in pyridine (2 mL), and the mixture was stirred at 80 °C for 3 h. The mixture was evaporated to dryness in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. Chromatography of the residue on silica gel (5 g) with ethyl acetate-hexane (8:92) as the eluent gave 15b (63 mg, 63%). Further elution with ethyl acetate-hexane (15:85) afforded 16b (20 mg, 18%). 15b: MS,  $m/e$  440 ( $M^+$ ), 380;  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  0.62 (3 H, s, H-18), 1.97 (3 H, s, Ac), 5.05 (1 H, m, H-3), 5.58 (1 H, s, H-7), 7.13 (1 H, s, H-19); IR ( $\text{CHCl}_3$ ) 2950, 1720  $\text{cm}^{-1}$ ; UV max (95% EtOH) 237.5 nm (log  $\epsilon$  4.22), 283 (4.29), 295.5 (4.17). 16b: MS,  $m/e$  500 ( $M^+$ ), 440, 380, 267;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.59 (3 H, s, H-18), 2.08

(6 H, s, Ac), 4.71 (2 H, s, H-19), 5.08 (1 H, m, H-3), 5.92 (1 H, s, H-7); IR ( $\text{CHCl}_3$ ) 2960, 1725, 1590  $\text{cm}^{-1}$ ; UV max (95% EtOH) 262 nm (log  $\epsilon$  4.08).

In a similar manner, 4a (112 mg) was treated with acetic anhydride-pyridine to afford 15b (83 mg, 70%) and 16b (24 mg, 18%).

**Reaction of Endoperoxides 3a and 4a with Triethylamine.** A solution of 3a (50 mg) and triethylamine (50  $\mu\text{L}$ ) in benzene (2 mL) was heated at 80 °C for 4.5 h. The mixture was evaporated to dryness, and the residue was chromatographed on silica gel (5 g) to give furan 15a (25 mg, 52%) and hemiacetal 13a (13 mg, 26%).

In a similar manner, 4a (50 mg) was treated with triethylamine to yield 15a (23 mg, 48%) and 14a (10 mg, 20%).

**Reaction of Endoperoxides 3a and 4a with CsF.** To a solution of 3a (45 mg, 0.11 mmol) in DMF (2 mL) was added CsF (16 mg, 0.11 mmol), and the mixture was stirred for 2 h at room temperature. The mixture was diluted with ethyl acetate, washed with water, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel (5 g) to give 15a (16 mg, 37%) and 13a (12 mg, 27%).

In a similar manner, 4a (30 mg) was converted to 15a (11 mg, 38%) and 14a (7 mg, 23%) on treatment with CsF in DMF.

**Reaction of Endoperoxides 3a and 4a with Boron Trifluoride Etherate.** To a solution of 3a (50 mg,  $1.2 \times 10^{-1}$  mmol) in benzene (2 mL) was added  $\text{BF}_3$ -etherate (16  $\mu\text{L}$ ,  $1.3 \times 10^{-1}$  mmol) at 0 °C. The reaction mixture turned to dark brown. After 25 min, ice chips were added, and the mixture was extracted with chloroform. The extract was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel (4 g) with benzene-hexane (1:3) as the eluent to give 17a: 28 mg (84%); MS,  $m/e$  278 ( $M^+$ ), 260, 165;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.61 (3 H, s, angular Me), 9.99 (1 H, s, CHO); IR ( $\text{CHCl}_3$ ) 2950, 1710  $\text{cm}^{-1}$ . For the semicarbazone: mp 160–161 °C; MS,  $m/e$  335 ( $M^+$ ), 276; UV max (95% EtOH) 232 nm. Anal. Calcd. for  $\text{C}_{20}\text{H}_{27}\text{ON}_3$ : C, 71.59; H, 11.12; N, 12.53. Found: C, 71.83; H, 11.13; N, 12.27.

In a similar manner, 4a (320 mg, 0.77 mmol) was treated with  $\text{BF}_3$ -etherate to yield 17a (145 mg, 68%).

**Reaction of Endoperoxides 3a and 4a with  $\text{ZnCl}_2$ .** A mixture of 4a (100 mg, 0.24 mmol) and  $\text{ZnCl}_2$  (10 mg,  $7.3 \times 10^{-2}$  mmol) in xylene (2 mL) was heated at 100 °C for 5 min. The mixture was directly chromatographed on silica gel (5 g). Elution with ethyl acetate-hexane (35:65) gave aldehyde 17a (57 mg, 85%).

In a similar manner, 3a (24 mg,  $5.8 \times 10^{-2}$  mmol) was treated with  $\text{ZnCl}_2$  to give 17a (13 mg, 81%).

**Isomerization of Aldehyde 17a to 17b.** A solution of 17a (10 mg) in ethanol (500  $\mu\text{L}$ ) was added to a solution of sodium ethoxide (prepared from 20 mg of Na) in ethanol (1 mL) at 0 °C, and the mixture was stirred at that temperature for 10 min. Ice chips were added, and the mixture was extracted with chloroform. The extract was washed with water, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was purified on TLC (silica gel, ethyl acetate-hexane 1:9) to give 17b: 9 mg (90%); MS,  $m/e$  278 ( $M^+$ ), 249;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.70 (3 H, s, angular Me), 9.53 (1 H, d,  $J$  = 3 Hz, CHO); IR ( $\text{CHCl}_3$ ) 2950, 1720  $\text{cm}^{-1}$ .

**Reaction of Endoperoxides 3a and 4a with Methanolic Aqueous HCl.** Concentrated hydrochloric acid (0.4 mL) was added to a solution of 3a (58 mg) in methanol (4 mL) at 0 °C. The reaction mixture turned gradually to dark brown. After 2 h at 0 °C, ice chips were added, and the mixture was extracted with chloroform. The extract was washed with water, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated. The residue was chromatographed on silica gel (5 g) with ethyl acetate-hexane (1:9) as the eluent to afford acetal 17c: 17 mg (38%); MS,  $m/e$  324 ( $M^+$ ), 322, 293, 292, 278, 261;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.67 (3 H, s, angular Me), 3.38 (3 H, s, MeO), 3.40 (3 H, s, MeO), 4.06 (1 H, d, acetal proton); IR ( $\text{CHCl}_3$ ) 2940  $\text{cm}^{-1}$ .

In a similar manner, 4a (74 mg) was treated with methanolic HCl to give acetal 17c (20 mg, 35%).

**Hydrolysis of Acetal 17c.** To a solution of 17c (17 mg) in acetone (2 mL) was added 18% aqueous hydrochloric acid (0.4 mL) at 0 °C, and the mixture was stirred at that temperature for 45 min. After dilution with water, the acetone was evaporated, and the aqueous residue was extracted with chloroform. The extract was washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated.

The residue was chromatographed on silica gel (3 g) to give 17b (18 mg, quantitative).

**Reaction of Endoperoxide 3a with Cobalt-Tetraphenylporphyrine.** To a solution of CoTPP (3 mg,  $4.5 \times 10^{-3}$  mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added a solution of 3a (20 mg,  $4.8 \times 10^{-2}$  mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) at  $-20^\circ\text{C}$ , and the mixture was stirred at  $-20$  to  $-10^\circ\text{C}$  for 5 h. Evaporation of the solvent and chromatography of the residue on silica gel (5 g) with ethyl acetate-hexane (80:20) as the eluent afforded 13a (15 mg, 75%).

**Reaction of Endoperoxides 3a and 4a with Tetrakis(triphenylphosphine)palladium.** A solution of 3a (35 mg,  $8.4 \times 10^{-2}$  mmol) and  $\text{Pd}(\text{Ph}_3\text{P})_4$  (10 mg,  $8.7 \times 10^{-3}$  mmol) in benzene (1 mL) was refluxed for 15 min. The dark red reaction mixture was directly chromatographed on silica gel to afford 15a (16 mg, 47%) (ethyl acetate-hexane, 20:80) and 13a (5.5 mg, 16%) (ethyl acetate-hexane, 70:30).

In a similar manner, 4a (50 mg) was treated with  $\text{Pd}(\text{Ph}_3\text{P})_4$  to give 15a (25 mg, 52%) and 14a (8 mg, 16%).

**(6*R*)-9,10-Secocholesta-5(10),7-diene-3 $\beta$ ,6,19-triol (9). Reduction of Endoperoxide 3a with  $\text{LiAlH}_4$ .** A solution of 3a (118 mg, 0.28 mmol) in dry ether (5 mL) was added to a suspension of  $\text{LiAlH}_4$  (22 mg, 0.58 mmol) in ether (2 mL) at  $0^\circ\text{C}$ . After 1 h at room temperature, the reaction was quenched with wet  $\text{Na}_2\text{SO}_4$ , and the mixture was filtered and washed with ethyl acetate-methanol (4:1). The combined filtrate and washings were evaporated, and the residue was chromatographed on

Sephadex LH-20 (10 g) with hexane-chloroform (35:65) as the eluent to afford triol 9: 83 mg (70%); MS,  $m/e$  418 ( $\text{M}^+$ ), 400, 382, 287, 269, 152, 134;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.50 (3 H, s, H-18), 3.88 (1 H, m, H-3), 4.05 (1 H, d,  $J = 12$  Hz, H-19), 4.25 (1 H, d,  $J = 12$  Hz, H-19), 5.14 (1 H, d,  $J = 8$  Hz, H-6 or H-7), 5.34 (1 H, d,  $J = 8$  Hz, H-7 or H-6); IR ( $\text{CHCl}_3$ ) 3610, 3410, 2960  $\text{cm}^{-1}$ .

**(6*S*)-9,10-Secocholesta-5(10),7-diene-3 $\beta$ ,6,19-triol (10). Reduction of Endoperoxide 4a with  $\text{LiAlH}_4$ .** Reduction of 4a (45 mg) with  $\text{LiAlH}_4$  was followed the procedure described above to give triol 10: 23 mg (51%); MS  $m/e$  418 ( $\text{M}^+$ ), 400, 287, 269, 153, 152, 135, 134;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.57 (3 H, s, H-18), 3.77 (1 H, d,  $J = 12$  Hz, H-19), 4.10 (1 H, m, H-3), 4.47 (1 H, d,  $J = 12$  Hz, H-19), 5.10 (1 H, d,  $J = 8$  Hz, H-6 or H-7), 5.52 (1 H, d,  $J = 8$  Hz, H-7 or H-6); IR ( $\text{CHCl}_3$ ) 3620, 3410, 2955  $\text{cm}^{-1}$ .

**Registry No.** 1a, 67-97-0; 1b, 50-14-6; 2a, 22350-41-0; 2b, 51744-68-2; 3a, 73047-69-5; 3b, 70779-98-5; 3c, 70779-97-4; 4a, 73047-65-1; 4b, 70779-99-6; 4c, 70801-88-6; 5a, 86728-02-1; 5b, 86728-03-2; 6a, 86728-04-3; 6b, 86728-05-4; 7a, 86728-06-5; 8a, 86728-07-6; 9a, 86832-43-1; 10a, 74532-19-7; 13a C(19)-(R), 86728-08-7; 13a C(19)-(S), 86728-09-8; 13b C(19)-(R), 86832-44-2; 13b C(19)-(S), 86832-45-3; 14a, 86782-90-3; 14b C(19)-(R), 86832-46-4; 14b C(19)-(S), 86832-47-5; 15a, 74546-09-1; 15b, 86728-10-1; 16b, 86728-11-2; 17a, 86728-12-3; 17a semicarbazone, 86728-13-4; 17b, 86728-14-5; 17c, 86728-15-6.

## Stereoselective Synthesis of (5*E*)- and (5*Z*)-Vitamin D<sub>3</sub> 19-Alkanoic Acids via Vitamin D<sub>3</sub>-Sulfur Dioxide Adducts

Sachiko Yamada, Takayoshi Suzuki, and Hiroaki Takayama\*

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

Katsuhito Miyamoto, Isao Matsunaga, and Yoshiharu Nawata

New Drug Research Laboratories, Chugai Pharmaceutical Co., Toshima-ku, Tokyo 171, Japan

Received March 1, 1983

(5*E*)- and (5*Z*)-vitamin D<sub>3</sub> 19-alkanoic acids 7 and 8 have been synthesized by a new method starting with vitamin D<sub>3</sub>. In this synthesis, sulfur dioxide was utilized innovatively to protect the *s*-cis diene part of vitamin D and at the same time to activate the terminal position (C-19) of the diene group for an electrophilic substitution reaction. The two C-6 epimers of the vitamin D<sub>3</sub>-sulfur dioxide adducts 2 and 3 were isolated in pure form, and the structure was determined unambiguously on the basis of X-ray analysis. The reaction of pure adducts 2b and 3b with *tert*-butyl  $\omega$ -iodoalkanoate 4 proceeded with complete regio- and stereoselectivity to afford 19-alkanoic acid derivatives 5 and 6 in which the substituent at C-19 is located *trans* to that at C-6. Thermolytic desulfonation of the 19-substituted adducts 5 and 6 in the presence of  $\text{NaHCO}_3$  afforded (5*E*)-vitamin D<sub>3</sub> 19-alkanoic acid derivatives 7 with high selectivity (ca. 93%), contrary to orbital symmetry rules. The (5*E*)-vitamin D derivatives 7 were converted to the corresponding (5*Z*)-vitamin D derivatives 8 in high selectivity (ca. 95%) by photosensitized isomerization.

Extensive studies on the metabolism of vitamin D<sub>3</sub> have lead to the discovery of more than 20 metabolites.<sup>1</sup> For clinical studies of the production of the biologically important metabolites, such as 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, 24(R),25-dihydroxyvitamin D<sub>3</sub>, etc., establishment of a sensitive, convenient, and selective analytical method has been needed. Radioimmunoassay has been highly successful for the measurement of steroid hormones. For use as an immunogen, a vitamin D molecule must be converted

to a derivative appropriate for combining with a protein. Recently, we have developed a new regioselective method of alkylating vitamin D at the 6- and 19-positions via its sulfur dioxide adducts 2 and 3.<sup>2</sup> In this method sulfur dioxide is used to protect the *s*-cis diene part of vitamin D, as well as to activate the terminal position of the diene group for electrophilic substitution reaction under basic conditions. We planned to apply the alkylation method to the synthesis of vitamin D<sub>3</sub> 19-alkanoic acid derivatives 7 and 8. The compounds 8 and 7 as components of a hapten are suitable derivatives for inducing antibodies for the radioimmunoassay of vitamin D and its 1 $\alpha$ -hydroxylated derivatives, respectively. Because the biologically essential hydroxyl group remains intact<sup>3</sup> in 7 and

(1) (a) DeLuca, H. F.; Schnoes, H. K. *Annu. Rev. Biochem.* 1976, 45, 631. (b) Takasaki, Y.; Suda, T.; Yamada, S.; Takayama, H.; Nishii, Y. *Biochemistry* 1981, 20, 1881. (c) Tanaka, Y.; Wichmann, J. K.; Schnoes, H. K.; DeLuca, H. F. *Ibid.* 1981, 20, 3875. (d) Wichmann, J. K.; Schnoes, H. K.; DeLuca, H. F. *Ibid.* 1981, 20, 7385. (e) Ohnuma, N.; Kruse, J. R.; Popjak, G.; Norman, A. W. *J. Biol. Chem.* 1982, 257, 5097. (f) Ohnuma, N.; Norman, A. W. *Ibid.* 1982, 257, 8261. (g) Yamada, S.; Ohmori, M.; Takayama, H.; Takasaki, Y.; Suda, T. *Ibid.* 1983, 258, 457.

(2) Yamada, S.; Suzuki, T.; Takayama, H. *Tetrahedron Lett.* 1981, 22, 3085.